Use of $[^{11}C]$choline PET-CT as a non invasive method for detecting pelvic lymph node status from prostate cancer and relationship with choline kinase expression

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Statement of Translational relevance

This study establishes the feasibility of using [11C]choline-PET CT as a non-invasive means of staging pelvic lymph nodes in high risk prostate cancer, being highly specific and more sensitive than PET alone or MRI including the detection of sub-centimetre disease. The high specificity could potentially be helpful clinically in terms of selecting out patients who may not require pelvic radiotherapy. We also showed, for the first time in prostate cancer biopsies that tumor radiolabelled choline uptake is related to choline kinase alpha expression in prostate cancer. This relationship could be exploited to develop new drug targets for prostate cancer.
Abstract

**Purpose:** To evaluate the accuracy and biological basis for $^{11}$C choline-PET-CT in the nodal staging of high risk localised prostate cancer patients.

**Experimental Design:** Twenty eight patients underwent dynamic $^{11}$C choline-PET-CT of the pelvis and lower abdomen prior to extended laparoscopic pelvic lymph node dissection (eLPL). The sensitivity and specificity of $^{11}$C choline PET, $^{11}$C choline PET-CT and MRI for nodal detection were calculated. Average and maximal standardized Uptake Values (SUV$_{\text{ave}}$, SUV$_{\text{max}}$) were compared with choline kinase alpha (CHKα) and Ki67 immunohistochemistry scores.

**Results:** 406 lymph nodes, in 26 patients, were assessable. 27 (6.7%) involved pelvic nodes at eLPL were detected in 9 patients. 17 out of the 27 involved nodes were sub-centimetre. The sensitivity and specificity on a per nodal basis were 18.5 % and 98.7%, 40.7% and 98.4 %, and 51.9% and 98.4% for MRI, $^{11}$C choline PET and $^{11}$C choline PET-CT, respectively. Sensitivity was higher for $^{11}$C choline PET-CT compared with MRI ($p=0.007$). A higher nodal detection rate, including sub-centimetre nodes, was seen with $^{11}$C choline PET-CT than MRI. Malignant lesions showed CHKα expression in both cytoplasm and nucleus. SUV$_{\text{ave}}$ and SUV$_{\text{max}}$ strongly correlated with CHKα staining intensity ($r=0.68$, $p<0.0001$ and $r=0.63$, $p=0.0004$, respectively). In contrast, Ki67 expression was generally low in all tumors.

**Conclusion:** This study establishes the relationship between $^{11}$C choline PET-CT uptake with choline kinase expression in prostate cancer and allows it to be used as a non-invasive means of staging pelvic lymph nodes, being highly...
specific and more sensitive than MRI including the detection of sub-centimetre disease.
Introduction

The evaluation of lymph nodes (LNs) has important therapeutic and prognostic significance in patients diagnosed with prostate cancer. Whilst a curative approach can be adopted for those with organ confined node-negative disease with modalities such as surgery, external beam radiotherapy or brachytherapy, those with node-positive disease ultimately relapse with metastatic disease (relapse rate 30-50% at 5 years, 90% at 10 years) (1, 2). As such, the presence of LN involvement reduces the 5 year disease free survival from 85% to approximately 50% with a shift in the focus of treatment to long-term androgen deprivation with the addition of pelvic radiotherapy to reduce loco-regional recurrence (3, 4). Pelvic LN dissection is currently the gold standard for evaluating the presence of nodal involvement (5, 6). This procedure can either be open or laparoscopic and is usually limited to the external iliac and obturator nodes, though a more extended procedure to include the internal iliac nodes is usually advocated for those with a higher risk of nodal disease (7). Either way, both these methods are invasive, associated with morbidity (8) and importantly may not be able to sample all potential LN areas.

It is thus important to have a sensitive and reliable non-invasive means of detecting nodal involvement. The criteria for nodal characterisation using cross-sectional imaging such as Computerized Tomography or Magnetic Resonance Imaging (CT or MRI) relies primarily on morphological assessment based on size and shape, with a nodal short axis diameter of 1cm generally accepted as an upper limit of normal. A threshold of 1cm in the short axis diameter for oval
nodes and 0.8cm for round nodes has been recommended as criteria for diagnosis of prostate cancer nodal metastases (9). A recent meta-analysis on the diagnostic accuracy of cross-sectional imaging in the staging of pelvic LNs in prostate cancer reported a high pooled specificity for MRI of 0.82 with a low and heterogenous pooled sensitivity of 0.39 (10). The lack of sensitivity belies the fact that nodal involvement is not always correlated with enlargement and enlarged nodes may also be due to a benign aetiology. Neither MRI nor lymphangiography has demonstrated higher sensitivity than CT scanning in the detection of nodal metastases (10, 11). The use of an MR contrast agent containing ultra-small particles of iron oxide (ferumoxtran10-Sinerem, USPIO) has been shown to yield sensitivity and specificity above 90% in the detection of prostate cancer LN metastases (12). However, this is not widely available and its intravenous infusion is not without side effects (13). Further studies using diffusion weighted MR whilst undoubtedly have improved intra-prostatic tumor detection and localisation, this method has been less satisfactory for assessing pelvic nodal disease (14).

Positron Emission Tomography (PET) offers functional information regarding tissue activity, thereby having the potential to provide superior staging information as well as the ability to monitor the response to treatment. The clinical experience with [18F]fluorodeoxyglucose (FDG) PET in prostate cancer is limited due to variable uptake of [18F]FDG in prostate cancer and the rapid excretion of FDG in urine, causing an accumulation of activity in the bladder (15-17).
[\textsuperscript{11}C]choline is a relatively new radiopharmaceutical for PET imaging and its utility in visualising and staging prostate cancer has been published (18, 19). Malignant transformation is postulated to be associated with changes in pathways of choline transport, utilization and increased CHK\(\alpha\) expression that will lead to an increased uptake of choline (20, 21). As illustrated by a number of MR Spectroscopy studies (21-23), CHK\(\alpha\) converts choline to phosphocholine in cells that is elevated during transformation and progression. The tumor PET signal from [\textsuperscript{11}C]choline, however, comprises of free [\textsuperscript{11}C]choline and [\textsuperscript{11}C]phosphocholine, as well as the oxidation product, [\textsuperscript{11}C]betaine (24). The PET signal (tumor [\textsuperscript{11}C]choline uptake) therefore, largely reflects transport and phosphorylation of [\textsuperscript{11}C]choline, and to a lesser extent (given that liver and kidneys produce most of the circulating [\textsuperscript{11}C]betaine), [\textsuperscript{11}C]choline oxidation. Unlike [\textsuperscript{18}F]FDG it has low renal elimination, and therefore visualisation of the prostate and surrounding nodes may be enhanced by the low accumulation of tracer within the bladder (16). Preliminary studies of [\textsuperscript{11}C]choline-PET in pelvic nodal staging in prostate cancer patients have shown early promise (25-27). However, no study to date has established a direct relationship between CHK\(\alpha\) expression and [\textsuperscript{11}C]choline uptake in prostate tumors.

This prospective study compares the use of [\textsuperscript{11}C]choline PET-CT with MRI in determining pelvic nodal status in patients with high risk localised prostate cancer undergoing surgical staging with eLPL (reference standard). We also sought to document the early kinetics of [\textsuperscript{11}C]choline from dynamic imaging up to 60 minutes post radiotracer injection. In addition, the association between
Materials and Methods

Patients

Patients with histologically confirmed prostate cancer staged as either high risk localised (either PSA >20ng/mL or Gleason score 8-10 or TNM stage ≥ T2) / locally advanced (nodal disease on staging MRI of the pelvis), were eligible for the study. Patients with visceral or bone metastases were ineligible. Ethical approval for the study was granted by the Hospital Research Ethics Committee. All patients gave written informed consent to participate in the study, which was carried out according to the Declaration of Helsinki guidelines. The administration of radioactivity for the PET scans was approved by the Administration of Radioactive Substances Advisory Committee, United Kingdom.

Imaging protocol

$[^{11}C] \text{choline}$ was synthesized at Hammersmith Imanet\textsuperscript{®} according to the method described by Pascali et al (28). To minimize post-biopsy effects, all imaging studies were performed at least 6 weeks after the transrectal biopsy. Subjects were asked to fast for 6 hours prior to the procedure (as bowel choline uptake interferes with interpretation of $[^{11}C] \text{choline}$ images). All patients were scanned on a PET-CT (GE-Discovery RX\textsuperscript{®}) scanner after being positioned such that the field of view (FOV) included the whole pelvis and the lower abdomen. This was
followed by a diagnostic quality CT scan (settings were; 300 mA, 120kVp, 0.8 sec/rotation i.e. 65 mA.s, 8 x 2.5mm slices and pitch 1.35) which was used for attenuation correction and co-registration with the PET images. $[^{11}C]$choline was administered by a bolus intravenous injection over 10 to 30 seconds. PET scanning (3-dimensional acquisition) was commenced over 2 bed positions (3 minutes per bed position) starting from the distal margin of the pelvic floor, covering the pelvis and lower abdomen (axial FOV per bed position, 15.7 cm; transaxial, 70 cm) for 65 minutes. Raw PET data were corrected for scatter and attenuation, and reconstructed with an iterative OSEM (ordered subset expectation maximum) algorithm comprising 8 iterations and 21 subsets. Decay corrected images were then viewed using Analyze® software (Analyze Version 7; Biomedical Imaging Resource, Rochester, MN, USA). From summed images, regions of interest (ROIs) were drawn manually around visible tumors in the prostate, and any visible pelvic nodes. The $[^{11}C]$choline radioactivity concentration within the ROIs was then determined and normalised for injected radioactivity and body weight to obtain $SUV_{ave}$ and $SUV_{max}$.

**MRI acquisition**

All patients underwent standard non-contrast staging MRI of the pelvis from aortic bifurcation to pubic symphysis comprising of T1-weighted axial images; axial, sagittal and coronal T2-weighted images and small FOV axial T2-weighted images through the prostate. The imaging was performed on a 1.5 Tesla Philips scanner in 5 patients and a 1.5 Tesla Siemens-Magnetom scanner in 21 patients.
Extended Laparoscopic extra-peritoneal Pelvic Lymphadenectomy (eLPL)

This was performed in a standard pre-defined protocol by the Urologists within an average of 22 days (2-49 days) of the \([^{11}\text{C}]\)choline PET-CT. Nodal status was discussed with the surgeon before lymphadenectomy using information from both MRI and the \([^{11}\text{C}]\)choline PET-CT images. The eLPL included nodes along the external and internal iliac vessels to the ureter proximally, obturator nerve medially and the genitofemoral nerve laterally. All nodes removed were carefully labelled for size and anatomical location. Nodes were fixed, paraffin embedded, stained with hematoxylin and eosin and reported as negative or positive for metastasis by a histopathologist with a specialist interest in urologic malignancy. The samples were also subjected to additional immunohistochemistry with Ki67 and CHK\(\alpha\) (\textit{vide infra}).

Image interpretation

The images of the \([^{11}\text{C}]\)choline PET-CT were interpreted prospectively in order to outline the ROIs, perform SUV analysis and discuss outcome with surgeons pre-operatively. Furthermore, all the imaging data (MRI, \([^{11}\text{C}]\)choline PET and \([^{11}\text{C}]\)choline PET-CT were pooled and evaluated by a dual accredited nuclear medicine radiologist, blinded to the results of the histopathology, on separate occasions to avoid reporting bias. The criteria used for assessing nodal involvement are given in Table-1.
**Immunohistochemistry**

CHK immunohistochemistry was performed using a primary polyclonal, human anti-CHKα antibody (catalogue No HPA024153, Sigma-Aldrich™, Dorset, UK) as per manufacturer’s instructions. The positive control used was bronchial tissue as per manufacturer’s specifications. Slides were then scored independently by 3 pathologists using the intensity of cytoplasmic and nuclear staining in prostate tumor cells from 1 to 3 (1+, low intensity; 2+, moderate intensity; 3+, high intensity including nuclear staining). Ki67 staining was performed using anti-Ki-67 antibody (NCL-Ki-67-MM1, Novocastra® Laboratories, Newcastle-upon-Tyne, UK). Tonsil tissue was used as a positive control. Ki67 score was determined by dividing the total number of Ki67 positive tumor cells with the total number of tumor cells counted in 8 high powered fields (200X magnification) using an Olympus microscope (Tokyo, Japan). The final score was expressed as a percentage.

**Statistical considerations**

The mean, standard deviation (SD), medians, range, and frequencies were used as descriptive statistics. The sensitivity, specificity and number of correctly recognized cases with MRI, [11C]choline PET and [11C]choline PET-CT in nodal detection were calculated for a per patient and per node analysis. The comparison of each imaging method was performed using the McNemar test implemented in its uncorrected exact form, based on the binomial distribution (29). Receiver operating characteristic (ROC) analysis and the area under the
curve (AUC) was determined by recalculating sensitivity and specificity for MRI, PET and PET-CT along the five-point grading scale for a per patient and a per nodal analysis using MedCalc statistical software (version 11.6.1, Mariakerke, Belgium). SUV$_{60,\,\text{ave}}$ and SUV$_{60,\,\text{max}}$ were compared with CHKα and Ki67 scores using Spearman’s correlation test and a p value of ≤ 0.05 was considered significant.

**Results**

**Patients**

28 patients underwent [$^{11}$C]choline PET-CT after fulfilling the inclusion criteria. Two patients could not undergo surgery after [$^{11}$C]choline PET-CT as one became unwell and the other changed his mind about undergoing surgery. Thus 26 patients underwent [$^{11}$C]choline PET-CT followed by eLPL/ sampling (one had LN sampling rather than dissection due to fibrotic and calcified lymph nodes). All patients subsequently had neo-adjuvant androgen deprivation followed by radical radiotherapy to the prostate and the pelvis. The median (mean; range) age of subjects was 67 years (67.7; 51 to 83 years), gleason score of primary prostate biopsies was 7 (7.6; 6-9) and the pre-treatment PSA levels were 26.25 (44.25; 8.1 – 209).

The interval between the [$^{11}$C]choline PET-CT and eLPL was an average of 22 days (2-49 days). From the 26 patients, a total of 406 pelvic LNs sampled were available for pathology, with a median of 16 (range 3-36) nodes harvested per patient. 27 (6.7%) involved pelvic nodes at eLPL were detected in 9 patients (Table-2). Of the involved nodes 17 out of the 27 LN were less than 10 mm in
size. The average nodal size of the histologically positive nodes was 9.8 mm with an average tumor focus of 5.7 mm.

The $[^{11}C]$choline PET-CT was well tolerated with no immediate or delayed complications observed.

**Time points for SUV measurement**

The average and maximum SUV at 60 minutes (SUV$_{60,\text{ave}}$ and SUV$_{60,\text{max}}$) were determined. Due to the rapid systemic metabolism of $[^{11}C]$choline (30), SUV has also been determined at an earlier time point. The time versus radioactivity curves (TAC’s) achieve a steady state after ~15 min (Supplementary Figure-1, 2). Hence SUV$_{15,\text{ave}}$ and SUV$_{15,\text{max}}$ were reported (Supplementary Figure-3).

**$[^{11}C]$choline uptake within the malignant prostate and pelvic nodes**

In addition to visualisation of nodal uptake, primary prostate tumors in all 26 patients were well visualised with good tumor-to-background ratios (Figure-1 and Supplementary Figure-4). The median (mean ± SD; range) SUV$_{60,\text{ave}}$ and SUV$_{60,\text{max}}$ were 4.85 (4.92 ± 1.75; 2.19-9.28) and 9.97 (11.05 ± 3.72; 4.73-20.54) respectively (median SUV$_{15,\text{ave}}$ and SUV$_{15,\text{max}}$ were 4.82 and 8.80 respectively). Dynamic TAC’s for $[^{11}C]$choline in primary prostate tumors and the nodal metastases demonstrated a good retention of activity after plateauing at ~15 min until 60 min with SUV$_{\text{ave}}$ (Supplementary Figure-1, 2). However, with SUV$_{\text{max}}$ there is a suggestion of increasing activity at 60 min which may be due to the contribution of $[^{11}C]$betaine.
Diagnostic performance of MRI, $[\text{^{11}C}]$choline PET and $[\text{^{11}C}]$choline PET-CT in detection of nodal disease

On a per patient basis, the sensitivity and specificity were 50 % and 72.2%; 66.7% and 76.4 % and 77.8% and 82.4% respectively for MRI, $[\text{^{11}C}]$choline PET and $[\text{^{11}C}]$choline PET-CT. On a per nodal basis the sensitivity and specificity were 18.5 % and 98.7%; 40.7% and 98.4 %; and 51.9% and 98.4% respectively for MRI, $[\text{^{11}C}]$choline PET and $[\text{^{11}C}]$choline PET-CT (Supplementary Table-1). No statistical difference between any two modalities was detected in the patient analysis, mainly owing to the relatively low number of subjects. In the per nodal analysis the sensitivity was significantly improved with the use of $[\text{^{11}C}]$choline PET-CT (p=0.007) and $[\text{^{11}C}]$choline PET (p=0.07) compared to MRI imaging, without a decrease in the specificity (p= 1, 1 and 0.48 for $[\text{^{11}C}]$choline PET versus MRI , $[\text{^{11}C}]$choline PET-CT versus MRI and $[\text{^{11}C}]$choline PET-CT versus $[\text{^{11}C}]$choline PET comparisons, respectively).

ROC analysis (Figure-2) showed the overall diagnostic performance improved in the following order MRI < $[\text{^{11}C}]$choline PET < $[\text{^{11}C}]$choline PET-CT.

Table-3 shows the detection rate of MRI, $[\text{^{11}C}]$choline PET and $[\text{^{11}C}]$choline PET-CT for nodal metastases according to the diameter of the infiltrated LNs. A higher LN detection rate, including the detection of sub centimetre nodes, was seen with $[\text{^{11}C}]$choline PET-CT than MRI. The mean diameter of the positive LNs on histopathology was 9.8mm and that of the true positive LNs was 13.8 and 9.4 mm, respectively, on MRI and $[\text{^{11}C}]$choline PET-CT (using CT component for size definition).
Sites of nodal involvement

The majority of the nodes were detected within the standard surgical template. However, four of 26 patients (15.4%) had focal increased uptake above the region of eLPL (Common Iliac (CI) region and lower para aortic region – median SUV$_{60\ ave}$ and SUV$_{60\ max}$ of 1.12 and 3.91; median SUV$_{15\ ave}$ and SUV$_{15\ max}$ of 2.61 and 6.51 respectively) as detected on imaging and therefore were not sampled. 8 out of 26 (31%) patients had nodes detected below the surgical template, out of which 3 patients had discrete unilateral uptake in the inguinal LNs (median SUV$_{60\ ave}$ and SUV$_{60\ max}$ of 1.21 and 2.50; median SUV$_{15\ ave}$ and SUV$_{15\ max}$ of 1.54 and 2.63 respectively); significantly lower as compared with true positive pelvic nodes (p-values of 0.002, 0.0002, 0.004 and 0.0002, respectively, for SUV$_{15\ ave}$, SUV$_{15\ max}$, SUV$_{60\ ave}$ and SUV$_{60\ max}$ in the two-sided t-test) which was interpreted as probably reactive uptake and therefore were considered non-metastatic (Supplementary Figure-5). One patient had a 5mm tumor focus in a genitofemoral node, which was outside the FOV.

Nodal Analysis on MRI

In 4 of 9 patients, MRI was positive for 5 malignant (true-positive (TP)) nodes with a median maximum diameter of 11 mm (range: 9 –21 mm; mean: 13.8 mm). In 22 malignant nodes using size criteria, MRI was false negative (FN). 18/ 22 (82%) nodes were sub-centimetre and were reported as normal. Four nodes > 10 mm were missed. This was due to a cluster of 3 nodes reported as one (Figure-
1), lateral extension of tumor obscuring the obturator node and 2 nodes measuring 12 and 15 mm on histology, which measured 4 and 5 mm on MRI, highlighting the pitfall of gross nodal measurements which may include surrounding peri-nodal fat and soft tissue.

In 4 patients, MRI was false-positive (FP) (a total of 5 nodes). This was due to a probable sampling error in 2 patients (Figure-1), reactive external iliac (EI) nodes in 1 patient and a positive round reactive obturator node which was negative on PET-CT.

**Nodal Analysis on $[^{11}\text{C}]$choline PET**

In 6 of the 9 patients, $[^{11}\text{C}]$choline PET alone was TP for 11/27 malignant LNs.

In the 16 FN malignant nodes, 13 were due to micro-metastases, 2 were mistaken for focal ureteric activity which was resolved with PET-CT and one node was in the saturation band (i.e. where there was an overlap when the 2 bed positions were fused). This saturation band, obscuring some parts of the imaged area is not a general feature of PET-CT but probably related to specific equipment settings or reconstruction.

In 4/17 patients $[^{11}\text{C}]$choline PET was FP (total of 6 nodes). There are varying reasons for this: one FP node was due to focal uptake in a calcified vessel mistaken for a node which was resolved with PET-CT; 2 nodes were reactive EI nodes; one node was situated in the saturation band and for the remaining nodes in 2 patients, there was a probable sampling error given that 15 and 28 nodes were removed in total from those patients respectively. The median SUV$_{15,\text{ave}}$:
SUV_{60, ave} and SUV_{15, max}: SUV_{60, max} of the false positive LNs were 2.54: 2.70 and 5.07: 6.51, respectively. SUVs of the TP LNs tended to be higher (median SUV_{15, ave}: SUV_{60, ave} = 2.99: 2.64 and SUV_{15, max}: SUV_{60, max} = 7.04: 7.77 respectively) than SUVs of the FP LNs although statistical significance was never reached (p-values of 0.48, 0.28, 0.56 and 0.22 for SUV_{15, ave}, SUV_{15, max}, SUV_{60, ave} and SUV_{60, max} in the two-sided t-test).

**Nodal Analysis on [^{11}C]choline PET-CT**

In 7/9 patients, [^{11}C]choline PET-CT was TP for 14 malignant LNs (Figure-1). The median maximum diameter of the malignant LNs detected was 9 mm (range: 4 – 20 mm; mean: 9.4 mm).

In 13 malignant nodes, [^{11}C]choline PET-CT was FN as explained in the preceding paragraph. In 3 patients, [^{11}C]choline PET-CT was FP in 6 nodes. In one patient, a further FP node close to the saturation band was called on PET-CT but not PET only. The other 5 nodes in two patients were FP on both PET-only and combined PET-CT as explained above.

**Ki67 and CHK-α expression in prostate tumors and nodal metastases**

Biopsy samples from 20 prostate cores and 7 metastatic nodes were available for immunostaining (Supplementary Table-2). There was cytoplasmic CHKα staining in all prostate tumor cells that varied in intensity from 1-3 (Figure-3a) compared to a positive control (Supplementary figure-6). In one section, some benign glandular areas were also weakly stained (Figure-3b). In one section an
increased nuclear staining for CHKα with increasing Gleason scores was also observed, especially between Gleason 3 and 5 (Figure-3c) visually differentiating the two grades. There was no relationship between cytoplasmic intensity and nuclear staining of CHKα. In fact, in one tumor, an area of prostatic-intraepithelial–neoplasia (PIN) showed cytoplasmic as well as nuclear staining (Figure-3d). In pelvic nodes, benign nodes showed no CHKα staining (Figure-3e) whereas malignant nodes showed moderate cytoplasmic staining (Figure-3f). Ki67 staining revealed (Figure-3g, 3h) that most primary and nodal prostate tumors had a low proliferation index (median 3%, range 1 to 17%).

Spearman’s correlation test was used to test the association between [11C]choline SUV and tumor immunohistochemistry, PSA and Gleason scores; (Supplementary Table-3). There was a positive correlation between SUV_{60, ave} or SUV_{60 max} (Supplementary Figure-7) with cytoplasmic CHKα intensity in prostate tumors (r=0.68, p<0.0001 and r=0.63, p=0.0004 respectively). This positive correlation was seen even at early time points (SUV_{15 ave}, SUV_{15 max}: r= 0.55, p=0.003 and r=0.46, p=0.02 respectively). [11C]choline SUV also weakly correlated with serum PSA levels at diagnosis. There was no correlation of [11C]choline SUV with either Ki67 or Gleason scores. The association between immunohistochemistry scores for CHKα and Ki67 with Gleason’s scores or PSA was assessed. Only Gleason scores and Ki67 indices showed a positive correlation (r=0.55, p=0.01).

Discussion
This study supports the feasibility of using $[^{11}C]$choline PET-CT in determining pelvic LN status in patients with high-risk prostate cancer. It is specific and shows early promise in yielding a greater diagnostic accuracy than either MRI or PET only scanning. This is especially evident in detecting sub-centimetre disease, although the sensitivity is not sufficient to exclude lymphadenectomy, as metastases <6 mm in particular may be missed. However, it has the potential to highlight nodal uptake outside the surgical template for LN dissection, especially in the CI and para-aortic area as demonstrated in this study, which can have significant consequences in terms of patient management.

The somewhat disappointing performance of $[^{18}F]$FDG PET in the setting of prostate cancer has prompted interest in newer PET tracers such as $[^{18}F]$ and $[^{11}C]$choline for the detection of primary tumor within the prostate and the staging of pelvic nodal disease. For the detection of the primary tumor, some authors have reported 100% sensitivity (19, 31, 32) while others report lower detection rates ranging from 19 – 58% depending on whether results were reported on a per patient or per lesion basis (33-36). Supplementary Table-4 summarises the published studies assessing LN stage, and shows varied and conflicting results (25-27, 37-39). Likewise for staging of pelvic nodal disease, the reported sensitivity and specificity ranged from 50 – 80% and 90 to 96% respectively in studies that employed PET alone based on a per patient analysis (25, 26). The variation in sensitivity may be in part due to patient selection.

In this study we have assessed the value of MRI, $[^{11}C]$choline PET and $[^{11}C]$choline PET-CT imaging in the preoperative staging of high-risk prostate...
cancer patients. We have shown an overall sensitivity and specificity of $[^{11}C]$choline PET-CT on a per patient basis, of 77.7\% and 82.4\% respectively in the detection of nodal metastases. These results were superior than both MRI (50\% and 72.2\%), and $[^{11}C]$choline PET (66.6\% and 76.4\%), though were not significantly different probably due to relatively low patient numbers. For MRI, the sensitivity & specificity achieved in our study are in keeping with previously reported data (12, 40). Dynamic contrast MRI may help with tumor localisation within the prostate but there are no specific reports on the additional benefit in nodal staging. More importantly on a per nodal basis (27/406), the sensitivity was significantly higher for $[^{11}C]$choline PET-CT (51.9\%) compared with MRI (18.5\%) (p=0.007) with a greater confidence for identifying sub-centimetre involved LNs, which occurred in 30\% of the cases. However, in this study apart from one 4mm node, we were unable to detect low volume metastases of less than 5mm in diameter, probably reflecting the limited spatial resolution of the current generation of scanners.

In one of the first published series, De Jong et al, obtained promising results with $[^{11}C]$choline PET in the preoperative nodal staging of 67 patients, with a sensitivity of 80\% in a per patient-based analysis. Metastatic LNs ranging from 0.5 – 3 cm in size with a mean SUV of 4.7 (2.9 – 9.1) were demonstrated. FP activity in 2 patients was attributed to inflammatory change and focal bowel activity. However, in their study, about 50\% of the node positive patients had a PSA of >50ng/mL (range: 3-500), compared to our mean PSA value of 44.25 ng/mL (range: 8.1-209), which may have contributed to a selection bias and may
under-represent the cohort of high risk localised prostate cancer patients for which radiotherapy to the pelvis would be indicated (25). Conversely Hacker et al (38) reported a very low sensitivity of 10% in a study of 20 patients assessed with F-18 fluorocholine. The mean diameter of metastatic lymph nodes in their study was 3.8mm which is well below the resolution of PET.

In more recent studies utilising [11C]choline PET-CT, Schiavina et al, (27) evaluated 57 intermediate or high-risk prostate cancer patients prior to surgical treatment. They reported a sensitivity of 60% and a specificity of 98% for the detection of nodal metastases. Husarik et al, (39) evaluated 111 patients with prostate cancer in a [18F]choline PET-CT study, 43 of whom had staging for assessment of primary disease. The PET-CT findings were correlated to the histo-pathological findings of 115 sampled LNs in 25 patients, with sensitivity & specificity on a per patient basis of 33% and 100% respectively.

Beheshti et al (37) evaluated 130 patients with intermediate or high-risk prostate cancer with [18F]fluorocholine (FCH) PET-CT prior to extended pelvic node dissection with sensitivity and specificity in the detection of malignant nodes of 45% and 96%, respectively. Furthermore they reported a change in management in 15% of cases. The authors also noted discrete FCH uptake in inguinal lymph nodes which was interpreted as probable reactive uptake and therefore excluded from data analysis. This was similarly observed in our study cohort (8 out of 26 patients), although the visible inguinal nodes had significantly lower SUVs than both the metastatic LNs and the malignant prostate. As nodal dissection does not routinely remove inguinal nodes as part of standard practice, it may be difficult to
ascertain if these were involved. The underlying assumption is that inguinal nodes were all within physiological limits of < 10 mm in dimension based on the fact that prostate tumors normally do not spread to inguinal nodes (41).

Two studies have reported on a per-nodal analysis. Husarik et al in their study including 25 patients staged with [18F]fluorocholine reported a low sensitivity of 20% (1 out of 5 involved nodes) and a specificity of 100%. All the FN nodes had tumor foci of < 5 mm. The mean SUV\textsubscript{max} of the detected LNs was 5.04; range 4.9–5.2). Notably only obturator nodes were removed rather than a more extensive lymphadenectomy and the authors did not comment on FCH positive nodes outside the obturator region. Schiavina et al, in the study mentioned earlier, reported a sensitivity of 41.4% and a specificity of 99.8% on a per nodal analysis. The mean diameter (in mm) of the metastatic deposit of true positive nodes was significantly higher than that of false negative nodes (9.2 vs. 4.2; \(p = 0.001\)). Our per-nodal results of sensitivity & specificity with [11C]choline were similar at 51.9% & 98.4%. A limitation of our study was the technical difficulties encountered with the interpretation of findings on the PET scans in the region of the saturation band (where there was an overlap when the 2 bed positions were fused) which accounted for some of the FP results on the PET alone. In the two patients in whom MRI and PET-CT were FP for a 26mm and 10mm node, there is the potential, despite careful use of surgical templates, that these nodes were not sampled. The median SUV\textsubscript{60, max} of FP LNs was 6.51, compared to 7.77 for the TP nodes.
This study is one of the first to evaluate the time dependent uptake of $[^{11}\text{C}]$choline in prostate tumors up to 60 minutes. Dynamic TACs for $[^{11}\text{C}]$choline in primary prostate tumors and the nodal metastases demonstrated a good sustained retention of activity after plateauing at ~15 min until 60 min with SUV$_{\text{ave}}$. However, with SUV$_{\text{max}}$, there is a hint of increasing activity at 60 min which may be due to the contribution of $[^{11}\text{C}]$betaine.

We showed for the first time in prostate tumor samples that tumor radiolabelled choline uptake is closely related to CHK$\alpha$ expression in prostate cancer. Both semi-quantitative parameters of choline uptake in tumors correlated well with CHK$\alpha$ scores (best with SUV$_{60, \text{ave}}$ $r=0.68$, $p<0.0001$, Spearman’s test). It was observed that benign prostatic tissue as well as PIN in the malignant cores showed cytoplasmic and nuclear staining. This may represent the range of CHK$\alpha$ expression in normal and pre-malignant tissues. In certain tissue sections, nuclear staining was observed, particularly in PIN and in certain high Gleason grade tumors and although we do not fully understand this phenomena, a possible reason is that, as with other cellular proteins like ERK1/2, phosphorylated CHK$\alpha$ may translocate to the nucleus. This hypothesis needs further evaluation. This study also showed that proliferation in prostate tumors was low, as reflected by the low Ki67 index in most tumors. This was contrary to the high CHK$\alpha$ expression. For this precise reason, there was no correlation between $[^{11}\text{C}]$choline SUV and Ki67 scores in tumors. A possible explanation for this is that for prostate malignancies, CHK$\alpha$ expression is a proliferation-independent marker of the prostate tumor phenotype. This is contrary to the
evidence in other cell/tumor types linking CHKα or choline metabolites and proliferation (42-44). Of note, one study has reported an association between choline uptake and Ki67 scores in prostate tumors (45). Piert et al showed that tumor-to-benign prostate background ratio was significantly high in tumors with a Ki67 score of > 5% (p<0.01). In our study, Ki67 indices were in the range of 1-17%. Seven cores had a Ki67 index of >5% with a mean SUV$_{60, \text{ave}}$ of 4.7 which is higher than that reported by Piert et al. Ki67 did, however, correlate with Gleason score (r=0.55, p=0.01, Spearman’s test).

The main drawback to $^{11}$C-choline is the relatively short half life (20.9 minutes) and thus the compound needs to be used close to where it is manufactured. Newer more stable and specific choline compounds are in development (24).

To conclude, this detailed study establishes the feasibility of $^{11}$C-choline PET-CT as a non-invasive means of staging pelvic LNs in prostate cancer, being highly specific (98.4%) and more sensitive than PET alone or MRI. The high specificity is potentially helpful clinically in terms of selecting out those patients with high risk prostate cancer who may not need pelvic radiotherapy. Although it cannot currently replace MRI as a staging tool, its ability to detect sub-centimetre nodes and a differential SUV value between involved and physiological LNs, allows for a functional imaging methodology for assessing the radiation response to involved nodes. The relationship between CHKα expression and $^{11}$C-choline uptake, together with the avid intra-tumoral uptake of choline demonstrated in this study, merits further investigation in a larger patient population and in patients with other risk profiles.
Acknowledgements

The authors would like to thank Ms Laura Maher for helping with patient recruitment, Dr David Pinato and Mr David Peston for advice on immunohistochemistry and Ms Kasia Kozlowski for editorial assistance. We would also like to thank the radiographers, radio-chemists, blood lab staff at Hammersmith IMANET® and finally all the patients who have taken part in this study.
References


### Table-1: Criteria for nodal involvement and ROC analysis

<table>
<thead>
<tr>
<th>Imaging Modality</th>
<th>Criteria for nodal involvement</th>
</tr>
</thead>
</table>
| MRI                    | **Size ratio criteria***(9, 12)*  
  **Benign:** nodes less than 8 mm short axis  
  **Malignant:** nodes > 10 mm short axis & Round nodes > 8mm (ratio of the short to long axis > 0.8 )                                                           |
| (¹¹C)Choline PET        | Focal uptake outside the normal physiological distribution of tracer in locations corresponding to nodal chains                                                       |
| (¹¹C)Choline PET-CT     | Nodes with increased tracer uptake above the background, even when <10mm in short-axis diameter                                                                       |

<table>
<thead>
<tr>
<th>Scale</th>
<th>MRI</th>
<th>(¹¹C)Choline PET/ PET-CT†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Nodes &lt;4mm or not seen</td>
<td>Definitely normal</td>
</tr>
<tr>
<td>2</td>
<td>Nodes = 4 – 5.9 mm</td>
<td>Probably normal (more likely to be physiological)</td>
</tr>
<tr>
<td>3</td>
<td>Nodes = 6 – 7.9 mm</td>
<td>Indeterminate (equally physiological/pathological)</td>
</tr>
<tr>
<td>4</td>
<td>Nodes ≥ 8mm but &lt;10mm</td>
<td>Probably abnormal (more likely to be pathological)</td>
</tr>
<tr>
<td>5</td>
<td>Nodes ≥ 10mm</td>
<td>Definitely malignant</td>
</tr>
</tbody>
</table>

**ROC –** Receiver operating characteristic curves.  
* Short-axis and long-axis diameters of the identifiable LNs were measured using electronic callipers on the scanner console.  
† Definitely normal, probably normal and indeterminate were considered benign and probably abnormal and definitely abnormal were considered malignant.
Table-2: Characteristics of patients with histologically positive nodes (9/26)

<table>
<thead>
<tr>
<th>Pt No</th>
<th>Age</th>
<th>GS</th>
<th>iPSA</th>
<th>cT</th>
<th>pN</th>
<th>No of + LN</th>
<th>Site of + LN</th>
<th>MRI</th>
<th>Size (mm)</th>
<th>PET</th>
<th>PET-CT</th>
<th>Size (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>73</td>
<td>7</td>
<td>8.54</td>
<td>T3a</td>
<td>N1</td>
<td>1</td>
<td>1-R Obt</td>
<td>R Obt</td>
<td>11</td>
<td>TP</td>
<td>1-R Obt</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>82</td>
<td>8</td>
<td>13.5</td>
<td>T3</td>
<td>N1</td>
<td>5</td>
<td>3-R Obt, 1-R II, 1-R GF</td>
<td>2-R EI</td>
<td>21.8</td>
<td>TP</td>
<td>3-R EI</td>
<td>20,11,7</td>
</tr>
<tr>
<td>56</td>
<td>7</td>
<td>50</td>
<td>T1c</td>
<td>N1</td>
<td>1</td>
<td>1-L Obt</td>
<td>R Obt</td>
<td>FN</td>
<td></td>
<td>FN</td>
<td>FN</td>
<td></td>
</tr>
<tr>
<td>65</td>
<td>9</td>
<td>209</td>
<td>T2b</td>
<td>N1</td>
<td>7</td>
<td>4-L EI, 3-R II</td>
<td>R II</td>
<td>19</td>
<td>TP</td>
<td>1-L EI, 3-R II</td>
<td>9,18,11,5</td>
<td></td>
</tr>
<tr>
<td>76</td>
<td>7</td>
<td>169</td>
<td>T4</td>
<td>N1</td>
<td>1</td>
<td>1-R Obt</td>
<td>L Obt</td>
<td>9</td>
<td>FP</td>
<td>R Obt</td>
<td>TP</td>
<td>9</td>
</tr>
<tr>
<td>76</td>
<td>7</td>
<td>21</td>
<td>T2b</td>
<td>N1</td>
<td>1</td>
<td>1-R Obt</td>
<td>FN</td>
<td>FN</td>
<td>FN</td>
<td>FN</td>
<td>FN</td>
<td>FN</td>
</tr>
<tr>
<td>61</td>
<td>9</td>
<td>45</td>
<td>T3</td>
<td>N1</td>
<td>8</td>
<td>1-R II, 2-R Obt, 3-L EI, 2-L Obt</td>
<td>FN</td>
<td>1-R Obt</td>
<td>TP</td>
<td>1-R Obt, 2-R II</td>
<td>6,6,4</td>
<td></td>
</tr>
<tr>
<td>76</td>
<td>9</td>
<td>24.5</td>
<td>T2b</td>
<td>N1</td>
<td>2</td>
<td>2-R EI</td>
<td>FN</td>
<td>FN</td>
<td>FN</td>
<td>FN</td>
<td>FN</td>
<td>FN</td>
</tr>
<tr>
<td>51</td>
<td>7</td>
<td>44.8</td>
<td>T3b</td>
<td>N1</td>
<td>1</td>
<td>1-L II</td>
<td>FN</td>
<td>10</td>
<td>TP</td>
<td>FN</td>
<td>L II</td>
<td>10</td>
</tr>
</tbody>
</table>

Mean 68.44 7.8 65.04 13.8 9.4
Median 73 7 44.8 11 9

GS-Gleason score; iPSA-initial prostate specific antigen; cT-clinical tumor stage; pN-pathological nodal stage; LN-lymph node; R-right; L-left; Obt-obturator; EI-external iliac; II-internal iliac; GF-genitofemoral; TP-true positive; FN-false negative; FP-false positive; + - positive.
Table-3: Detection rate of the 3 imaging modalities by the size of the node

<table>
<thead>
<tr>
<th>Size of infiltrated nodes (mm)</th>
<th>No of Lymph nodes (LN)</th>
<th>MRI + (%)</th>
<th>[11C]choline PET + (%)</th>
<th>[11C]choline PET-CT+ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 – 1.9</td>
<td>1</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>2 – 4.9</td>
<td>4</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (25)</td>
</tr>
<tr>
<td>5 – 9.9</td>
<td>12</td>
<td>0 (0)</td>
<td>4 (33)</td>
<td>4 (33)</td>
</tr>
<tr>
<td>≥ 10</td>
<td>10</td>
<td>5 (50)</td>
<td>7 (70)</td>
<td>9 (90)</td>
</tr>
</tbody>
</table>

+ - Positive
Legends to Figures

**Figure-1:** a; T1 weighted MRI (i), Axial $[^{11}\text{C}]$choline PET (ii), CT (iii), and PET-CT fused (iv) shows focal uptake in a 4mm right obturator node (arrowed) clearly separate to the ureter on the PET only (ii). In retrospect visible on MRI (i) but not called as well below size criteria, b; Coronal T2 weighted MRI (i), $[^{11}\text{C}]$choline PET Maximum Intensity Projection (MIP) (ii), CT (iii), and PET-CT fused (iv) shows focal uptake in cluster of right external iliac nodes (arrowed). Note uptake in prostate extending to seminal vesicle (green arrow on coronal MIP), c; T1 weighted MRI (i) axial $[^{11}\text{C}]$choline PET (ii), CT (iii), and PET-CT fused (iv) shows focal uptake in 10 mm left obturator node (arrowed) which was false positive.

**Figure-2:** ROC curve analysis showed the area under the curve to be 0.625, 0.820 and 0.830 respectively for MRI, $[^{11}\text{C}]$choline PET and $[^{11}\text{C}]$choline PET-CT on a per patient basis and 0.677, 0.745 and 0.766 respectively on a per nodal analysis. The overall diagnostic performance improved in the following order MRI < $[^{11}\text{C}]$choline PET < $[^{11}\text{C}]$choline PET-CT.

**Figure-3:** CHK immunostaining showing a; cytoplasmic staining, b; CHKα expression in benign (B) and malignant (M) acini, c; differential CHKα in Gleason stage 3 and 5, d; CHKα in PIN, e; no staining in a benign node, f; malignant focus in node, g; low staining for the Ki67 antibody in prostate tumor and, h; low staining for Ki67 antibody in metastatic node. Magnifications of 200X.
Figure 1
Per Patient basis

AUC-0.625

AUC-0.820

AUC-0.830

AUC-0.677

AUC-0.745

AUC-0.766

Figure 2
Figure 3
Use of [11C]choline PET-CT as a non invasive method for detecting pelvic lymph node status from prostate cancer and relationship with choline kinase expression

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